bio-protocol Preprint

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Organoid viability assay

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An abbreviated version of this protocol was published in eLIFE in Nov 2020

A fully automated high-throughput workflow for 3D-based chemical screening in human midbrain organoids

DOI: 10.7554/eLife.52904

Detailed protocol

Dear Maheen Iqbal,
thank you for your interest in our research and methods. I would like to refer you to our publication titled "A fully automated high-throughput workflow for 3D-based chemical screening in human midbrain organoids" in eLife available under open access terms. Here, we detail the reagents, volumes, and incubation times you requested. In reading through this article, you will also see that we are using whole, non-dissociated organoids for this protocol, and that we are not using embedding matrices including matrigel. I will paste the relevant method section below. If you require additional information, please get in touch via the corresponding author information.
Thanks!
Jan Bruder

from our eLife article:
Organoid viability assay
To measure the viability of individual organoids, we used the CellTiter-Glo 3D Cell Viability Assay (Promega) according to the manufacturer's instructions. The entire procedure was performed using an automated liquid handling system (Beckman Coulter) and is thus fully scalable and HTS-compatible. In short, the reagent and the AMOs were brought to room temperature in their 96-well culture plates for 30 min and the media volume of each 96-well was adjusted to 55 µl. We added an equal volume (55 µl) of the CellTiter Glo 3D reagent and let it shake on a Thermomixer (Eppendorf) at 900 rpm for 5 min before incubating the samples protected from light at room temperature for 25 min. To prevent cross-talk between wells when measuring the luminescence, we next transferred the contents from the clear 96-well culture plates to opaque white 384-well Lumitrac plates (Greiner) with two technical replicates per sample. Luminescence was recorded immediately after transfer with a Synergy Mx plate reader (BioTek). The results were outputted to Microsoft Excel, reformatted and then transferred to GraphPad Prism v8.4.2 for plotting. Coefficients of variation (CVs) were calculated via CV = standard deviation/mean.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Schöler, H. R. and Bruder, J. (2022). Organoid viability assay. Bio-protocol Preprint. bio-protocol.org/prep1681.
2. Renner, H., Grabos, M., Becker, K. J., Kagermeier, T. E., Wu, J., Otto, M., Peischard, S., Zeuschner, D., TsyTsyura, Y., Disse, P., Klingauf, J., Leidel, S. A., Seebohm, G., Schöler, H. R. and Bruder, J. M.(2020). A fully automated high-throughput workflow for 3D-based chemical screening in human midbrain organoids. eLIFE. DOI: 10.7554/eLife.52904